In vivo modulation of CD1 and MHC class II expression by sheep afferent lymph dendritic cells. Comparison of primary and secondary immune responses

IN VIVO MODULATION OF CD1 AND MHC CLASS II EXPRESSION BY SHEEP AFFERENT LYMPH DENDRITIC CELLS

Comparison of Primary and Secondary Immune Responses

By JOHN HOPKINS, BERNADETTE M. DUTIA, RAYMOND BUJDOSO, AND IAN McCONNELL

From the Department of Veterinary Pathology, University of Edinburgh, Edinburgh EH9 1QH, Scotland

In this article we detail the kinetic changes that take place in the expression of immunologically important cell surface molecules by lymph-borne cells during in vivo immune responses and describe the consequences of these changes for the immunological functions of these cells. We used the unique advantages of the cannulated lymphatic model in the sheep (1) to integrate the physiological changes occurring in antigen-stimulated lymphoid tissue with lymph cell function. This experimental model has the potential to provide information on lymphoid and accessory cell function that is not readily gained with other systems. The approaches we have used involve the development of mAbs to molecules associated with immune cell function, the use of these monoclonals to measure the changes in immune cell phenotype that occur during in vivo immune responses, and correlation of these changes with alteration of in vitro immunological functions of afferent lymph dendritic cells (DC).[1]

The major advantage of the cannulated lymphatic model lies in the opportunity to access in vivo fractionated cell populations from distinct lymphoid compartments. To do this, the afferent lymphatics to, and the efferent lymphatics from single peripheral lymph nodes are cannulated and lymph is collected quantitatively over extended periods of time (often 3–4 wk).

The cellular constituents of afferent lymph and efferent lymph are different (2). Afferent lymph contains few cells ($0.5–1 \times 10^6$/ml) but does include between 1 and 10% dendritic cells and <10% B cells. Efferent lymph contains 5–10-fold more cells, consisting of ~30% B cells and 70% T cells (3). No cells of the macrophage/monocyte or DC series occur in efferent lymph. Evidence for this comes from morphological observation (2) as well as from data that show that efferent cells are incapable of responding to soluble antigen in vitro without the addition of accessory cells (4). Experiments described in a companion paper (5) demonstrated that DC isolated

This work was supported by the Medical Research Council (MRC grant 822698) and by the Agriculture and Food Research Council (LRG 31). Address correspondence to Dr. J. Hopkins, Department of Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh EH9 1QH, Scotland.

[1] *Abbreviations used in this paper:* BCG, Bacille Calmette Guerin; DC, dendritic cell; PPD, purified protein derivative of tuberculin.

from sheep afferent lymph are able to associate with antigen injected in vivo and to present that antigen to antigen-specific T cells. Furthermore, they seem to have a unique role in the induction of primary immune responses (6). In afferent lymph these cells can be defined as those with dendritic morphology that constitutively express MHC class II and CD1 (5, 7). The major biological function of class II is known to be the binding of antigenic peptides and communication with the TCR (8) and CD4 (9) molecules on T helper/inducer populations.

The CD1 family of molecules is expressed mainly by cortical thymocytes, B cells, and dendritic cells in several sites (10). Within the thymus CD1 is thought to be covalently associated with the CD8 molecule and it is speculated to be involved in T cell selection (11). The function of CD1 expressed on DC is at present unknown.

We document the alteration of MHC class II and CD1 expression by dendritic cells in sheep afferent lymph draining sites of both primary and secondary in vivo antigen challenge. Using in vitro MLR and soluble antigen-induced proliferation (of antigen-specific T cell lines) assays we have studied the accessory function of these cells isolated at intervals during immune responses. Increased accessory function of DC correlates both temporally and quantitatively with MHC class II expression but not CD1.

Materials and Methods

Animals and Surgery. 1-yr-old blackface × finn crossbred sheep were obtained from IAGPR, Dryden, UK. Sheep were primed by intradermal injection of five human doses of Bacillus Calmette Guerin (BCG; Glaxo, Greenford, UK) and intramuscular injection of 1 mg OVA or sperm whale myoglobin (both Sigma Chemical Co., Poole, UK) in CFA. Prefemoral afferent (peripheral) lymph was collected by the ablation of the prefemoral lymph node and cannulation of the efferent duct 6 wk later (3). This duct now contains afferent lymph and is known as a "pseudoafferent." Efferent lymph was collected by the chronic cannulation of the popliteal efferent duct (12). This procedure is necessary because the yields from standard afferent lymphatic cannulations are always very low. Animals were allowed at least 7 d postoperative recovery before the start of the experiments. Lymph was collected quantitatively in sterile (siliconized) bottles containing heparin.

Cell Fractionation. Afferent lymph cells were fractionated into lymphocytes and dendritic cells by centrifugation over stepped metrizamide (Nygaard & Co., Oslo, Norway) gradients (13). Purity of fractionation was always assessed by staining with the monoclonal anti-sheep CD1 antibody, VPM 5 (5), as well as by using geimsa-stained cytospin smears. B and T cells were enumerated using FITC-conjugated mAbs to sheep λ light chains (VPM 8) and the sheep analogue of CD5, SBU-T1 (7). Efferent lymph T cells were isolated by nylon-wool (Type 200; Fenwall Laboratories, Deerfield, IL) fractionation (14).

Flow Cytometry. Class II and CD1 quantitation by flow cytometry was done using single batches of FITC-conjugated SW73.2 or VPM 5 at 10 μg/ml in HBSS/0.1% BSA/0.01 M sodium azide. $F(ab')_2$ of SW73.2 and IgM of VPM 5 were coupled to FITC at a wt/wt ratio of 100:1, protein/FITC). SW73.2 is a rat mAb specific for a nonpolymorphic determinant on sheep class II that reacts with the known products of all the expressed class II loci (15). 10^6 cells of each cell population (afferent lymph dendritic cells and lymphocytes) were stained daily with 50 μl FITC-antibody. After a 30-min incubation at 4°C the cells were spun through 0.5 ml FCS, washed once, made to 0.5 ml with HBSS + 5% FCS, then fixed by the addition of 0.5 ml 1% paraformaldehyde. Daily samples were stored at 4°C for analysis at the end of the experiment. 10^4 cells were analyzed by flow cytometry using a Becton Dickenson & Co. (Mountain View, CA) FACS IV with a 488-nm argon laser. FITC (520 nm) emission was detected with the photomultiplier tube voltage set at 555 mV. Forward and side (90°) scatter amplification was linear, while FITC amplification was logarithmic (all 256 channels). Antibody-negative cells were gated by reference to fixed cell samples reacted with a negative

mAb. Within experiments, class II-positive controls were made by the daily staining of 2×10^5 BL20 cells, a cloned bovine leukosis cell line (16). In all experiments BL20 had a modal channel number of 180 (178–186) when stained with FITC-SW73.2 as detailed above. The data are expressed as relative fluorescence intensity (modal channel number). The cell cycle state of the cells was assessed by using propidium iodide (PI; Sigma) using the methods of Vindelov et al. (17). Cell couplets were gated out by using resting efferent lymph cells as the control.

Saturation Binding Studies. The quantitation of anti-class II antibody binding sites per cell was by saturation binding as described previously (15, 18). Briefly, washed cells were made to 2×10^6/ml in RPMI 1640 + 10% FCS + 0.01 M sodium azide. 250 μl of cell suspension were mixed with doubling dilutions of ^{125}I- (Radiochemical Centre, Amersham, UK) labeled Fab' SW73.2 at 4°C. After a 2-h incubation, aliquots of the reaction mixture from each dilution were layered on to 200 μl phthalate (dibutyl/dionyl phthalate at a ratio of 4:1) in 0.4 ml microfuge tubes and centrifuged at 13,000 *g* for 5 min then snap frozen in liquid N_2. The frozen microfuge tubes were then cut in order to separate the radioactivity associated with the cells from the unbound antibody. As with the flow cytometry experiments, these studies were controlled daily by measurements on the BL20 cell line (mean of 2×10^5 antibody binding sites per cell, variation $1.6–2.3 \times 10^5$). The proportion of class II-positive cells in each population was always considered when calculating the number of antibody binding sites per cell.

Purification of Monoclonal Ig. IgM from VPM5 ascitic fluid (and of the mouse IgM anti-sheep class II mAb VPM 3) was purified by initial precipitation with ammonium sulphate at 45% saturation and then gel filtration using Sephacryl S300 (Pharmacia Fine Chemicals, Piscataway, NJ). IgG_{2a} and $F(ab')_2$ isolation from SW73.2 ascitic fluid were as described previously (15). The Fab' fragment was produced from the $F(ab')_2$ by reduction (10 mM dithiothreitol in 100 mM Tris, pH 8, for 1 h) and alkylation (30 mM iodoacetamide for 1 h). The $F(ab')_2$ was purified on DEAE-cellulose (10 mM phosphate buffer, pH 7.5). The Fab' fragment was purified by gel filtration on Sephadex G200 superfine (Pharmacia Fine Chemicals). Purified Ig of VPM 5 and $F(ab')_2$ of SW73.2 for the proliferation assays was made to 100 μg/ml in RPMI 1640 + 10% FCS before sterile filtration and storage at −70°C. Supernatants of an IgM anti-sheep class II (VPM 3), the anti-sheep CD1 monoclonal SBU-T6 (7) and the anti-bovine CD1 antibodies CC13 and CC14 (19) used in the in vitro assays were dialyzed against RPMI 1640, filter sterilized, then FCS was added to 10%.

Production of Antigen-specific Cell Lines. Cell lines specific for the antigens OVA and purified protein derivative of tuberculin (PPD) were constructed as described by Bujdoso et al. (20). Briefly, efferent lymphocytes were cultured at 2×10^6/ml in 24-well culture plates with RPMI 1640 + 10% FCS containing either OVA or PPD at 25 μg/ml. Irradiated PBM were added to 5×10^5/ml. After 7 d incubation, viable cells were harvested by LYMPHOPREP and further incubated for 14 d at 1×10^5/ml with 20 U human rIL-2 (a gift from Biogen SA, Basel, Switzerland) added every 3 d. Viable cells were again harvested and restimulated with antigen by culturing at 5×10^5 cells/ml with 1×10^6/ml irradiated, autologous PBM. After 7 d the cells were again harvested and expanded with IL-2 for 14 d. The viable cells were then cryopreserved in liquid N_2 using a programmed cell freezer (Planar Products Ltd., London, UK).

In Vitro Proliferation Assays. Briefly, 10^4 antigen-specific T cells per well (in round-bottomed microtest trays; Gibco-Biocult, Paisley, Scotland) were cultured with OVA, sperm whale myoglobin (both Sigma Chemical Co.) or PPD (batch 297; Central Veterinary Laboratories, Weybridge, UK) at a final concentration of 25 μg/ml. APCs for soluble antigen-induced lymphocyte proliferation and MLR assays were 1×10^3 to 2.5×10^4 PBMC or afferent lymph DC irradiated with 3,000 rad γ-irradiation. PBMC were prepared by centrifugation over LYMPHOPREP (Nygaard). Responder cells in MLR assays were 2.5×10^4 allogeneic efferent lymph T cells purified by nylon-wool.

The effects of monoclonal anti-class II and anti-CD1 antibodies on in vitro proliferation and MLR assays were assessed by the addition of 20 μl of SW73.2 and VPM 5 at dilutions of 0.01–100 μg/ml. Supernatants of VPM 3, SBU-T6, CC13, and CC14 were used at final concentrations of 1/2, 1/5, and 1/10.

Experimental Protocol. A prefemoral "pseudoafferent" lymphatic was cannulated and 7 d later antigen was introduced into the drainage area by intradermal injection of 50 μg PPD, OVA, or sperm whale myoglobin. Daily for 2 d before and for 8 d after in vivo antigenic challenge in both antigen-naive and in antigen-primed animals the levels of both MHC class II and CD1 expression by the purified DC were assessed by flow cytometry. Quantitative expression of class II expression was also measured by a saturation binding technique. The immunological function of the DC was assessed by testing their ability to present soluble antigen (either OVA or PPD) to autologous OVA-specific or PPD-specific T cell lines and to stimulate allogenic efferent T cells in an MLR. The involvement of MHC class II and CD1 in the alterations in DC function was studied by examining the effects of mAbs to these molecules in these in vitro assays. 11 sheep were used (four with PPD and four with OVA and three with sperm whale myoglobin) to assess secondary antigenic challenge; while six (two for each antigen) sheep were used to compare the primary and secondary responses.

Results

Effect of In Vivo Antigenic Challenge on Afferent Cell Output. The changes in the percentage of class II-positive lymphocytes in afferent lymph during a secondary immune response to PPD can be seen in Fig. 1 *a*. The proportion of sIg^+ cells remains unaltered at ~9% (6–12%) of the total, but class II-expressing T cells increase from

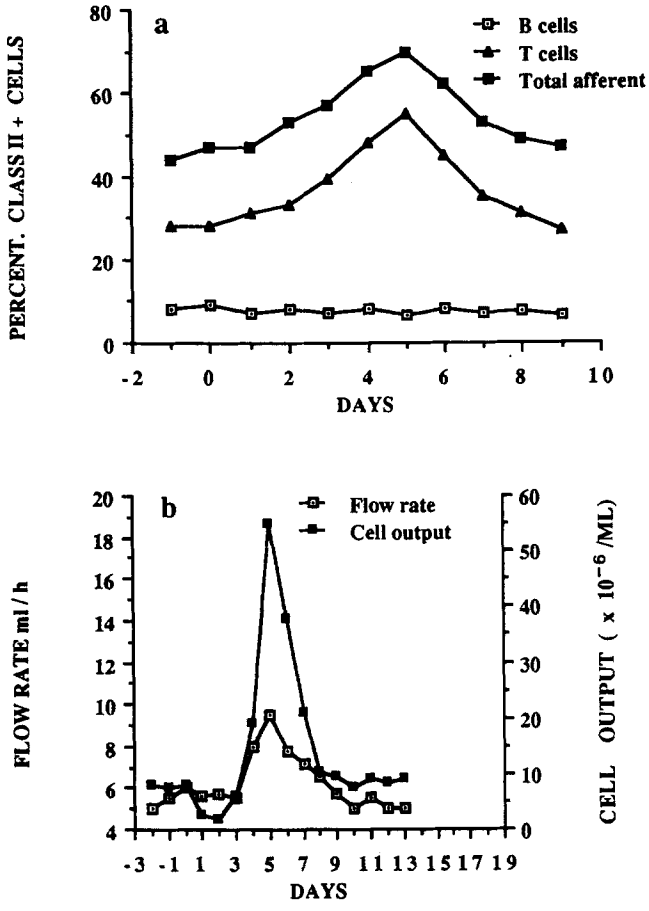

FIGURE 1. Alterations in afferent lymph cell kinetics during a secondary immune response in vivo. (*a*) Output of MHC class II-positive cells in afferent lymph draining sites of antigen inoculation, in primed sheep. (*b*) Changes in total cell output and lymph flow rates.

~28% of the total (variation 23–35%) up to 54% (45–59%). The proportion of $CD1^+$ DC (1–10%) and macrophages (<0.2%) within individual animals remains stable throughout the response. The results obtained with OVA and myoglobin were the same as those with PPD.

Local antigenic stimulation also induces changes in lymph cell kinetics (Fig. 1 *b*). Resting afferent lymph has a total cell output of between 6 and 10×10^6 cells/h, this falls three- to fivefold, to $<2 \times 10^6$/h during days 1–3 because of a drop in lymph cell concentration. By day 5 the cell output increases >5-fold (to $5–8 \times 10^7$/h), partly because of a rise in lymph fluid flow rate. These changes are only transient however, as by 8–9 d after challenge the lymph cell phenotype and kinetics are similar to those in resting lymph. These changes are restricted to secondary responses. No detectable modulation of class II expression and cell kinetics occur during primary antigenic challenge in antigen-naive sheep.

Alteration in Afferent DC Expression of Class II. The rise in the proportion of class II-positive cells in afferent lymph during secondary responses is also accompanied by marked increases in the overall level of class II expression by single cells. Antibody binding studies have shown that DC constitutively express $\sim 3 \times 10^5$ MHC class II molecules per cell (assuming one antibody binding site per class II molecule) (variation between 2.3 and 3.6×10^5), which doubles to $\sim 6 \times 10^5$ on day 3, and increases almost sixfold to 1.7×10^6 ($1.5–2.1 \times 106$) in response to in vivo challenge with antigen (Fig. 2 *a*). These figures were calculated by saturation binding, where 1×10^6 DC were reacted with dilutions of ^{125}I-labeled $F(ab')_2$ SW73.2, 1 μg antibody is 150,000 cpm. 1×10^6 DC from day 0 afferent labeled with 7,000 cpm at saturation (i.e., 3×10^5 molecules per cell), on day 3 they labeled with 15,000 cpm at saturation (6×10^5 molecules per cell), and on day 5 with 41,000 cpm at saturation (1.7×10^6 molecules per cell). Cellular class II expression by lymphocytes is also increased, rising from a mean resting level of $<1 \times 10^5$ ($0.5–1.3 \times 10^5$) up to 2.3×10^5 ($1.6–2.6 \times 10^5$). These alterations are only transient, however (Fig. 2 *b*), as quantitative class II expression of afferent cells returns to resting levels by day 8 after challenge.

An exact parallel series of experiments has been done to monitor the changes, in terms of both cell output and class II expression, in the primary response. Using sperm whale myoglobin as antigen and repeating the experiments as described above we were unable to demonstrate any significant alteration in either cell number or MHC class II expression. In contrast, subsequent secondary challenge with myoglobin in the same animals gave identical results as those described for OVA and PPD.

Flow cytometry confirms these data (Fig. 3). DC from unstimulated lymph (time 0) have a log relative fluorescence intensity (modal channel number, MCN) of 196 (190–205); this increases to MCN of 255 (>250 in all of 10 experiments) on days 4 and 5 after in vivo challenge, and returns to 194 (190–202) on day 8. The class II-positive lymphocytes from unstimulated lymph have a MCN of 133 (125–138), which increases to 164 (159–170) days 4 after antigen (data not shown). An increase of 30 channels represents a doubling of fluorescence intensity, therefore the rise in MCN of DC from 196 (on day 0) to >250 (on day 5) represents at least a fourfold increase in class II expression.

Alteration in Afferent DC Expression of CD1. CD1 expression by DC in resting afferent lymph show a single population (time 0) with a MCN of 187 (variation between

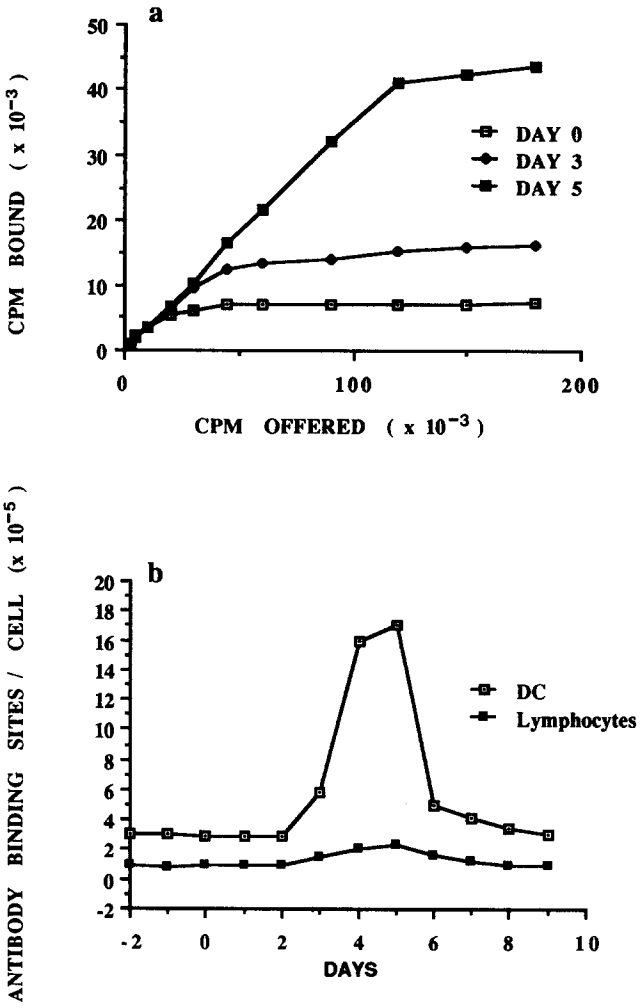

FIGURE 2. Quantitation of MHC class II expression by afferent lymph DC isolated at different times after secondary in vivo antigenic stimulation. (*a*) Saturation binding curves of 10^6 DC from days 0, 3, and 5 incubated with ^{125}I-labeled F(ab')$_2$ SW73.2. (*b*) Quantitative variations in MHC class II expression by DC isolated at daily intervals during a secondary immune response, as measured by saturation binding analysis.

179 and 196 in five experiments). Antigenic stimulation of an antigen-naive animal (primary stimulation) results in the appearance of three distinct CD1 expressing populations of DC at the 14-h time point (Fig. 4). The dull staining population consists of 15–20% of DC, with MCN of 183 (175–188); the intermediate population of 52–60% has MCN of 205 (201–209) and the bright population of 15–20% with MCN of 233 (228–239). The alteration in CD1 expression is only transient as the flow cytometry profile by 45 h is identical to that at time 0.

In contrast, changes in CD1 expression after antigenic challenge in a primed sheep (secondary stimulation) are far less transient and show the appearance of the intermediate CD1 expressing population with MCN of 207 (197–214) from 2–6 d after antigen inoculation (data not shown). Although the DC have distinct levels of CD1 expression (after both primary and secondary challenge), they have a homogeneous expression of class II and cannot be distinguished by their 90° scatter profile (side

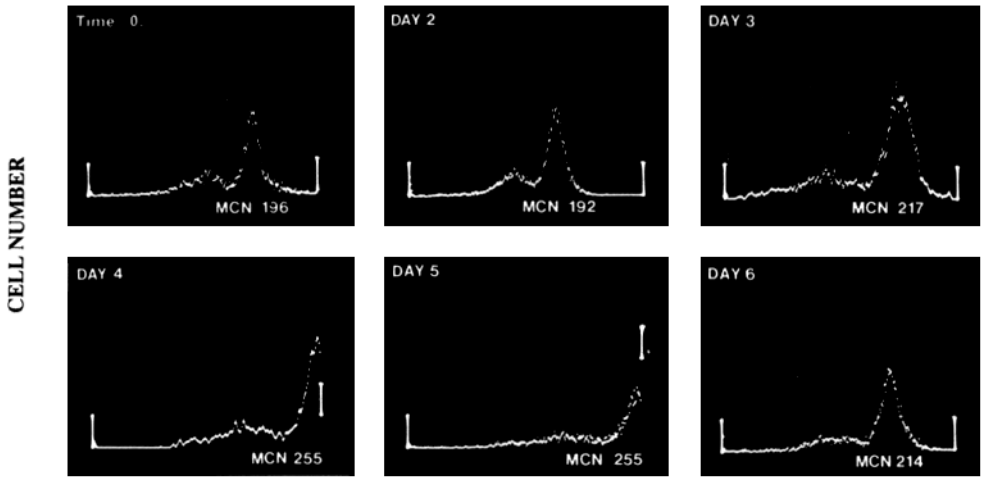

FIGURE 3. Flow cytometry analysis of purified afferent lymph DC isolated at daily intervals after secondary in vivo antigenic stimulation. Each panel shows a fluorescence histogram of DC stained with FITC-SW 73.2 (anti-sheep class II). The figures represent the modal channel number (MCN) of the antibody-positive cell population. 520 nm emitted light was detected with PMT set at 555 mV, amplification was logarithmic (256 channels).

scatter). The increase in class II expression during secondary challenge occurs with every cell and not on minor populations.

By coupling mAb staining with cell cycle analysis it is possible to correlate cell surface phenotype with stages of cell activation. Staining with PI and the monoclonals SW73.2 (anti-class II) and VPM 5 (anti-CD1) showed that the increase in DC class II expression and change in CD1 phenotype was not associated with these cells entering cell cycle (data not shown).

Alteration in the In Vitro Accessory Function of DC as a Result of In Vivo Antigenic Stimulation. In the companion article (5) we have reported that in vivo pulsed afferent DC are capable of stimulating autologous T cell lines, in the absence of exogenous antigen, in an antigen-specific manner. In this article we investigate their accessory potential in the presence of exogenous antigen. DC fractionated from afferent lymph were tested both before, and at daily intervals after, in vivo antigenic challenge to see whether their antigen-presenting ability was altered and if such alteration correlated with changes in the expression of either CD1 or MHC class II. Antigen-induced proliferation of PPD- and OVA-specific T cell lines and MLR assays were used to assess the in vitro antigen-presenting function of the DC. In all these experiments irradiated PBMC were used as a control for fractionated DC. Representative data from these experiments are shown in Figs. 5 and 6.

Fig. 5 compares the effects of in vivo primary and in vivo secondary antigenic challenge (to sperm whale myoglobin) on the ability of DC, isolated at different times after immune stimulation, to present alloantigen to allogeneic T cells and OVA to an OVA-specific T cell line. The same sheep were used for both the primary and secondary experiments and before the start of the secondary responses the animals

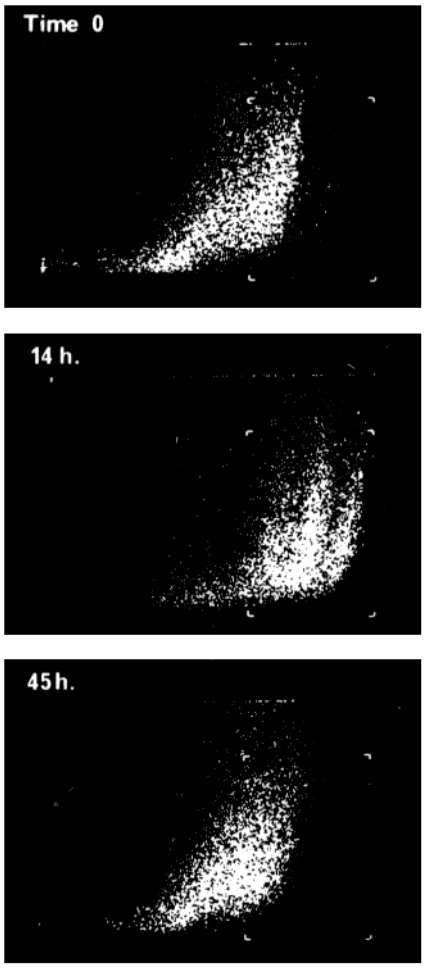

FIGURE 4. Flow cytometry dot plots (linear side [90°] scatter vs. $\log_{10}$ fluorescence intensity) of afferent lymph DC stained with the anti-sheep CD1 mAb VPM 5. Cells are taken at intervals after primary antigenic challenge in an antigen-naive animal.

were primed with, and shown to be responsive to, sperm whale myoglobin. It is clear from these data that the capacity to induce T cell proliferation by DC isolated during the in vivo primary responses does not vary significantly after antigen inoculation. In contrast is the effect of secondary in vivo challenge. Although the results with PBMC are unchanging, the proliferation of the T cell lines and allogeneic efferent cells cultured with DC is markedly increased in both the antigen-induced proliferation and MLR assays respectively.

Fig. 6 further illustrates the ability of DC from in vivo stimulated afferent lymph, isolated during a secondary response to myoglobin, to stimulate in an MLR or OVA-induced proliferation assay. It is clear that DC induce a substantially greater proliferation than PBM, which is most noticeable at limiting accessory cell concentrations. 1×10^3 DC, isolated 4 and 5 d after in vivo challenge with myoglobin, support

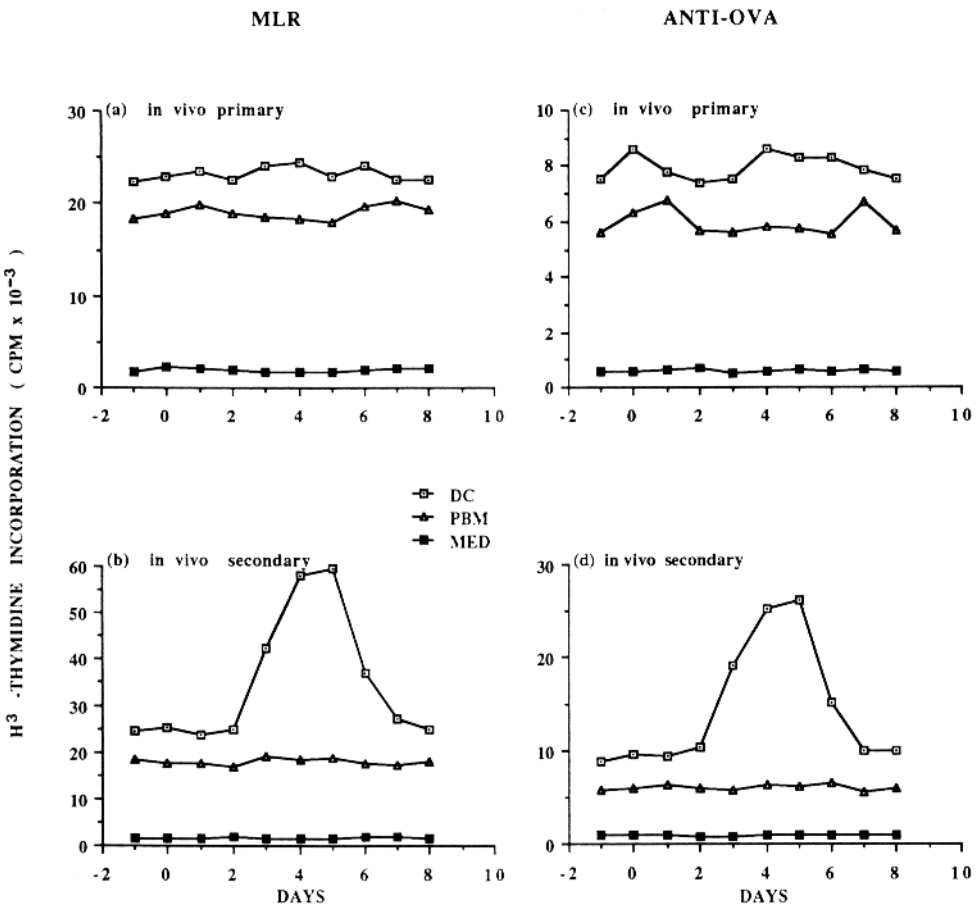

FIGURE 5. Comparison of the accessory function of afferent lymph DC and PBMC isolated at daily intervals after in vivo primary of in vivo secondary antigenic challenge with sperm whale myoglobin. Response of 2.5×10^4 efferent lymphocytes in MLR assays (*a* and *b*); and 1×10^4 OVA-specific T cells OVA-induced proliferation assays (*c* and *d*). 1×10^4 accessory cells were used in both assay systems.

18–20,000 cpm (MLR) and 15–16,000 cpm (OVA) proliferation, approximately fivefold greater than that initiated by DC from resting lymph (4,000 and 3,000 cpm, respectively). That number of PBM did not induce proliferation in either assay. The modulation of proliferation induced by DC correlates temporally with the differential expression of MHC class II and not CD1. The relative degree of in vitro function gradually declines to preinoculation levels by day 8. Identical results were obtained when PPD and OVA were used as the in vivo antigen and also when autologous efferent lymphocytes were used as responder cells in the antigen-induced proliferation assays. These data support the view that DC, activated during secondary antigenic challenge, have an increased capacity to present exogenous antigen.

Effect of Monoclonal Anti-Class II and Anti-CD1 Monoclonals on In Vitro DC Function. To assess the role of class II and CD1 expression on the antigen-presenting function

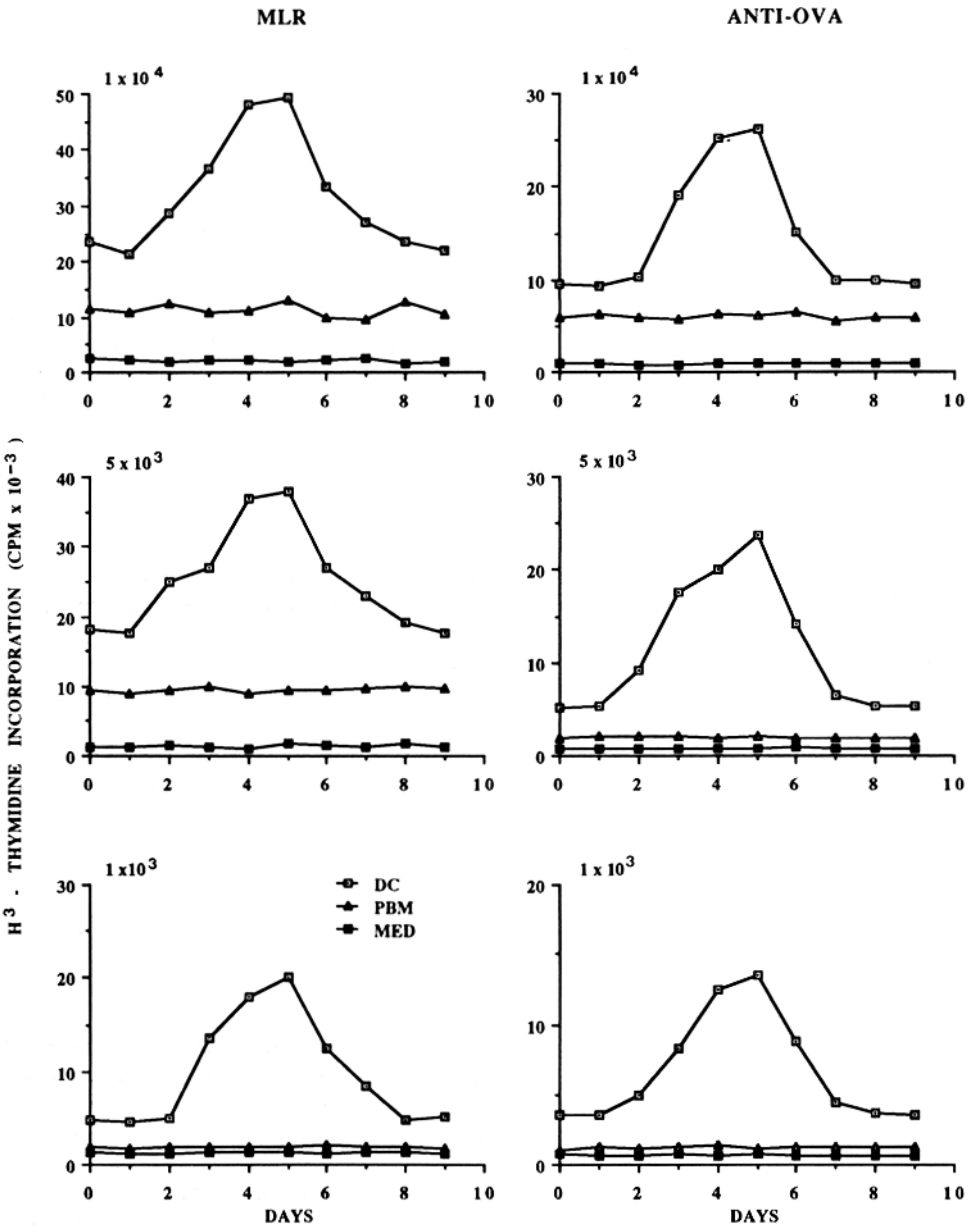

FIGURE 6. Response of 2.5×10^4 efferent lymphocytes in MLR assays; and 1×10^4 OVA-specific T cells in OVA-induced proliferation assays. Titration of PBM and afferent lymph DC isolated at intervals after in vivo challenge with myoglobin in a myoglobin-primed sheep (secondary immune response).

of afferent lymph DC, titrations of purified Ig [or $F(ab')_2$ of SW73.2] of the anti-class II and anti-CD1 monoclonals were added to the in vitro assays. The anti-class II antibodies tested, SW73.2 (IgG2a) and VPM 3 (IgM), totally inhibited lymphocyte proliferation in both assays, with DC taken from resting afferent lymph.

The enhanced proliferation found with the use of DC isolated at days 3, 5, and 8 after secondary antigenic challenge was also blocked. SW73.2 and VPM 3 gave 50% inhibition at 10 ng/ml, and at a 1/10 dilution, respectively, with 100% inhibition at 10 μg and at a 1/2 dilution (data not shown). The addition of the anti-CD1 antibodies VPM 5, SBU-T6, CC13, and CC14 had no effect at any time on either assay, even with supernatant dilutions as high as 1/2.

Discussion

In this article we examine the kinetic changes in afferent lymph cell output and phenotype that occur as a result of in vivo primary and in vivo secondary antigenic stimulation in the drainage area of the afferent lymphatic, and correlate these changes with alterations in in vitro immunological function of afferent lymph DC. Afferent lymph DC are defined as those cells of dendritic morphology that constitutively express both MHC class II and the sheep analogue of CD1 (5).

Primary antigenic challenge stimulates no detectable alterations in afferent cell output or the numbers of cells expressing sIg, class II, or CD1, although dramatic changes occur in levels of CD1 expression by a large majority of individual DC. Resting afferent lymph has a relatively homogeneous population of $CD1^+$ DC with a MCN of 187. Within 14 h of primary challenge up to 60% DC express 1.5 times the number of CD1 molecules (MCN of 205) and 15–20% express almost 3 times the level (MCN of 233). These changes are very transient however, as by 45 h the pattern in resting lymph is re-established.

In contrast are the changes that occur during secondary responses. Although the relative proportions of the different cell populations (DC, B cells, and T cells) remain stable, the total cell output is decreased on days 1–3 after challenge but is increased to approximately fivefold preinoculation levels on days 4–7. In addition, the proportion of class II–positive cells increases, resulting in a more than doubling of the proportion of class II–positive cells at days 4–5. This results from a rise in the number of class II–positive T cells as there is no change in the proportion of DC and B cells in afferent lymph. Unlike the primary response, no immediate effect on CD1 expression is seen, but the intermediate CD1-expressing population (MCN 207) appears by day 2, reaching up to 35% on day 5 and disappearing after day 7.

In vivo antigenic stimulation in primed animals was demonstrated to have profound effects on the quantitative expression of MHC class II, as well as CD1. If we assume one antibody binding site per class II molecule, the expression of class II on DC is increased from 3×10^5 molecules (channel number 196) to 1.7×10^6 molecules (channel number >250) per cell as a result of antigenic challenge. This level of class II expression by sheep DC and its relative increase induced by exogenous stimuli are very similar to those shown for mouse epidermal Langerhans' cells (21) which seem to be the precursors of lymphoid DC (22). Changes in class II expression by afferent lymphocytes are less marked, increasing from $\sim 1 \times 10^5$ (channel number 133) to 2×10^5 (channel number 164). These data establish that the stimuli for the increase in class II occur in antigenically primed animals only. This strongly suggests that they arise from antigen-activated T cells. Cytokines from activated T cells have been shown to induce class II synthesis (23). Candidate mediators therefore would include BSF-1/IL-4 and IFN-γ, both of which have been shown to induce class II expression in nondividing cells (24–27). It is of interest to note that the rela-

tive increase in class II expression by DC (×5–6) is much greater than for afferent lymphocytes (×2). This raises the possibility that the class II expressed by DC and lymphocyte populations are qualitatively different. Afferent T cells for instance could be restricted in their expression of individual locus products. The alternative explanation, that the increase in class II expression is a result of cell division (28), has been eliminated, as we have shown that the afferent lymph DC are not in cycle. There have been no previous descriptions of quantitative variations of CD1 expression; the stimuli for the changes in CD1 expression are, therefore, open to speculation. The data in this article illustrate that afferent lymph DC of the sheep, like those of the rat (29), rabbit (30), pig (31), and bovine (32), can present soluble antigen to antigen-specific T cells as well as stimulating allogeneic lymphocytes in the primary MLR. The capacity of afferent DC to enhance in vitro lymphocyte proliferation seems also to correlate directly with increased class II expression but not with CD1. At day 5 after in vivo antigen, the DC express five- to sixfold the preinoculation levels of class II and, at limiting DC concentrations, are about fivefold as active in the proliferation assays. The main increase in dendritic cell expression of CD1 occurs by day 2, before any increase in class II and enhancement of antigen presentation. In addition, treatment of the cells with anti-class II antibodies, but not anti-CD1 antibodies, abrogates all in vitro accessory function, including that at the peak of the in vivo response.

Although class II expression is necessary for antigen presentation (33, 34) and cells such as macrophages and astrocytes can present antigen only after they have been induced to express class II (35, 36), it is beginning to be clear that quantitative variation of expressed class II plays a central role in immune regulation (37–40) and aberrant expression may contribute to autoimmune disease (41). These data support the proposition that the magnitude of T cell responses is related to the product of the concentrations of antigen and class II (42). The function of CD1 expressed by DC is unclear, but in view of its differential expression during both primary and secondary responses, as well as its homology with both MHC class I and class II (43), it is intriguing to speculate on its potential as an antigen-binding protein. In this article we document a temporal correlation between enhanced accessory function of DC and increases in class II expression, but antigenic stimulation almost certainly affects parameters other than CD1 and class II synthesis. IL-1, for instance, is produced as a result of many different stimuli (44) and has been shown to upregulate the stimulatory activity of DC (45). In vivo stimulation could induce DC to secrete IL-1 or even express membrane IL-1 on the cell surface (46). The results could also be explained by the fact that the enhanced capacity to induce a proliferative response in lymphocytes is mediated by IL-1 or similar molecules released by in vivo activated DC.

In summary, we describe in this article our approach to the study of the molecules associated with immune cell function. We describe the in vivo variation of CD1 and class II expression by DC, implicating quantitative variations of class II expression in immune regulation in vivo. We also extend our knowledge to include those events that occur within the relevant physiological environment in vivo. Further work will concentrate on the differential expression of the products of the individual CD1 and class 11 genes by the distinct cell populations within the sheep lymphoid system.

Summary

The experiments described in this article characterize the phenotypic and functional changes in afferent lymph cell populations that occur as a result of in vivo immune stimulation. During the primary immune response (in antigen-naive sheep) there are very transient increases in level of CD1 expression by subpopulations of dendritic cells (DC) but no alterations in cell kinetics or MHC class II expression. In contrast, secondary antigenic challenge (in primed sheep) into the drainage area of an afferent lymphatic causes profound changes in the cell output, characterized by a greater than threefold drop in total cell output on days 1–3 followed by an approximate fivefold rise on day 5. There is also a substantial increase in both the proportion of MHC class II–positive T lymphocytes (from 28 to 54%) and in the quantitative expression of class II by both DC and lymphocytes. Class II expression by DC increases five- to sixfold by day 5, while the level of expression of class II on lymphocytes approximately doubles. The increase in CD1 expression during the secondary response is more prolonged than during the primary response, being detectable between days 2 and 6 after challenge. The rise in class II affects the whole DC population, in contrast to CD1 where the increase affects only a subpopulation of cells.

In terms of functional properties, afferent lymph DC isolated during a primary response show no alteration of their activity, whereas DC taken 4–5 d after secondary challenge are up to fivefold more active in their ability to present soluble antigen to primed autologous T cells and to antigen-specific cell lines as well as to stimulate in the MLR. The relative expression of class II correlates temporally with an increased capacity of DC to present antigen. Monoclonal anti-class II antibodies totally inhibit the in vitro assays but anti-CD1 antibodies have no effect. The previous paper (5) has demonstrated that afferent DC can associate with antigen in vivo and can present that antigen to antigen-specific T cells. This article extends our knowledge of DC biology and demonstrates that DC, activated during secondary in vivo immune responses, have an enhanced ability to present an antigen, unrelated to that used for challenge, to specific T cell lines. This enhancement correlates directly with quantitative variation of expressed class II and not CD1 and suggests that this variation in class II expression plays a physiological role in in vivo immune regulation.

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